[Reprinted from *American Medicine*, Vol. V, No. 11, pages 412-415, March 14, 1903.]

ON TRANSPLANTATION OF TUMORS.[1]

BY

LEO LOEB, M.D.,

of Montreal, Can.

From the J. H. R. Molson Laboratories of Pathology and Bacteriology, McGill University, Montreal.

1.—ON THE RESISTING POWER OF EXCISED PIECES OF TUMORS.

In several former communications I reported the results of a series of transplantations of a cystic sarcoma and of a mixed tumor, a sarcocarcinoma of the thyroid.[2] In these investigations I used the method of transplantation (1) to determine experimentally the conditions of growth of tumors, and (2) to approach certain questions of the structure of tumors. I also reported some experiments in which I tried to determine the resisting power of excised pieces of tumors to unfavorable conditions.

Since then these latter experiments have been continued and I now wish to report upon the results obtained, mentioning also some previous observations. In these experiments I used in part tumors derived from the sarcomatous part of the mixed tumor of the thyroid; mainly, however, tumors derived from a third sarcoma of the thyroid of a rat, which was cystic, like the first sarcoma.[3]

I. *Experiments to Produce Tumors by Transplanting Excised Pieces of the Sarcoma which had Previously Been Kept at a Temperature of 3° C. to 4° C.*—After I had succeeded in successfully transplanting pieces of tumor, which after the death of the animal had been kept at room temperature for 12 hours, I further extended the time of keeping the tumor outside of the living animal before transplanting it.

Pieces of a large tumor, which was infected in the

---

[1] A somewhat fuller report will appear later.

[2] (*a*) On Transplantation of Tumors, Journ. Med. Research, n. s. Vol. i, No. 1, 1902. (*b*) Uber Transplantationen eines Sarcoma de Thyreoidea bei einer weissen Ratte, Virchow's Archiv, Vol clxvii. (*c*) Further Investigations in Transplantation of Tumors, Journ. Med. Research, Vol. iii, 1902.

[3] I am indebted for this tumor to the kindness of Professor Herzog, of Chicago. The experimental part of this work was partly done in the Gratwich Research Laboratory of Buffalo in the summer of 1902.

center, having been kept on ice for 24 hours, were transplanted into seven animals, three of which developed tumors. One piece, kept at room temperature for the same time, only produced suppuration.

Tumors kept on ice for two days resulted, after transplantation, in the growth of two tumors. Pieces kept in the thermostat for the same time did not grow. Pieces kept on ice for five days produced, after transplantation; three tumors.

In the summer of 1902 a series of further experiments was made in which other parts of the third cystic tumor of the thyroid were kept from 1 to 28 days at a somewhat higher temperature, at about 10° C. Transplantation of such pieces, or an injection of a suspension of small particles of the sarcoma in normal salt solution, gave without exception no result. Control pieces, however, which were transferred at once, resulted in the majority of cases in the successful development of tumors. In one case, for instance, four out of five transplanted pieces, in another five of six transplanted pieces, grew.

The cause of the want of success in these latter experiments probably consisted either in the somewhat higher temperature at which these pieces had been kept, or in the fact that only very small pieces were transplanted. I had observed in my second series that under unfavorable conditions, in which infection of the piece is present, the size of the transplanted piece is of importance.

II. *Experiments on the Influence of Heat on Excised Pieces of Tumor.*—Experiments to determine the maximum temperature to which tissues can be exposed without losing the power to grow after transplantation have not to my knowledge been recorded. It was therefore of especial interest to try such experiments with sarcomas, it being *a priori* uncertain if they would behave like ordinary tissues. In these experiments pieces were cut out of transplanted tumors and either minced at once with sterile 0.7% sodium chlorid solution and then heated in a sterile dish on the water bath, or the whole piece was first heated and afterward transplanted as a whole or minced in salt solution and injected.

Pieces kept 40 minutes at a temperature of 43° C. to 44° C. and a piece kept for 25 minutes at a temperature of 43° C. and afterward 15 minutes at a temperature of 45° C., all grew, but pieces kept for 30 minutes at 45° C., as well as pieces exposed to a still higher temperature, up

to 50° C., for 30 minutes, did not grow in a single instance. In all eight series of such experiments were made. Seven rats were inoculated with pieces of tumor kept at 43° C. for 30 to 40 minutes, and of these seven, four developed tumors. Of the other three cases, in one rat the inoculation was without success, in another the tumor developed after inoculation, but later ceased to grow, in the other the microscopic examination showed only the presence of some doubtful nodules.

In the unsuccessful cases in which pieces were kept for 30 minutes at a higher temperature than 43°C. to 44° C., simultaneous control transplantations with the same, but not heated material, were to a large extent successful. On April 18, for instance of two control experiments one was successful; on May 27, of six control experiments, five were successful; on May 29, of four control experiments, three were successful; on June 13, of six control experiments, two were successful.

One might have expected that pieces previously heated would either have been made entirely ineffective by the heating, or if their power to grow had not been destroyed by the heating, would under the favorable circumstances in which they are situated after injection in a living rat, recover in a short time and grow just as well as the piece previously not heated. This, however, was not the case. All tumors formed after transplantation of pieces previously heated, were markedly weakened in their growth. They began to grow later than the control tumors, and after having started to grow, the rate of their development was slower; growth practically ceased after about five to eight weeks. The power of the transplanted pieces to produce tumors was therefore weakened by the heating. Three of these tumors were examined microscopically.

III. *The Influence of Glycerin on Excised Pieces of Tumors.*—Pieces which were kept after extirpation of the tumor for 17 to 24 hours in glycerin, either on ice or at room temperature, were transplanted after having been for a variable period washed with sterile normal salt solution. They were then minced in a 0.7% solution of sodium chlorid and injected in different experiments into seven animals. One developed a large and another a small tumor, while a third,[1] injected intraperitoneally, also

[1] In this case the possibility is not absolutely excluded that the animal in which this tumor developed had been previously inoculated with a piece of tumor which had been kept for 24 hours in chloroform water, their being a possibility of an interchange of animals.

developed a large tumor. (Injection of the material was both subcutaneous and intraperitoneal.)

For instance, in a successful series of this nature of July 7, of 13 *control* rats injected with fresh tumor seven developed tumors. Of the two pieces put into glycerin and implanted into rats one gave rise to a tumor.

Already in former experiments, in which the mixed tumor of the thyroid was used, pieces had been put into glycerin before transplanting them. These experiments could not be finished at the time; in two cases, however, when pieces had been kept in glycerin seven days and one-half day, respectively, they were transplanted into rats and taken out for microscopic examination one day later. Microscopically the nuclei of the cells showed in many places an irregular arrangement of the chromatin, otherwise the structure of the tumor was well preserved. In the piece put into glycerin for the shorter period mitoses were present, due perhaps to the migration of cells into this piece.

The number of positive results with glycerin being small, it will be necessary to continue these experiments further until more definite conclusions can be drawn.

IV. *Growth of Pieces of Tumor Which Before Transplantation Were Put Into Solutions of Potassium Cyanid.* —It was of interest to see whether pieces of mammalian tumors which had been kept in a solution of normal 1–700 to normal 1–1,000 potassium cyanid outside of the body were capable after transplantation of leading to the formation of tumors, it being known that potassium cyanid inhibits the growth of bacteria, and a tumor which has been transferred through so many generations of animals being usually no longer free of infection from saprophytic organisms. In several of the experiments the tumors used were too severely infected to give positive results. In other cases the control experiments made with immediate transplantation of tumors did not give any positive results. In one control series five tumors grew, but the pieces previously kept in potassium cyanid did not grow. In two other cases, however, in which the control experiments were also positive, the pieces kept in potassium cyanid did grow. In one case a piece developed a tumor after having been kept for 24 hours in normal 1–1,000 potassium cyanid solution. Among three control animals two developed tumors, one did not.

In the second successful series four rats were injected

with a suspension of minced tumor which had previously been kept for 40 hours on ice in a normal 1–700 solution of potassium cyanid, made up with physiologic (0.75%) sodium chlorid solution. In this last experiment three rats developed tumors. Among eight control transplantations six were positive.

In these cases again it was apparent that the pieces previously treated were not as efficient to produce tumors as the ones transplanted immediately; they began to grow some days later, and grew at a slower rate.

In all cases before the injection of suspension the potassium cyanid solution was removed from the piece by washing with normal salt solution.

2.—ON THE INFLUENCE OF BACTERIAL PRODUCTS ON THE GROWTH OF TRANSPLANTED PIECES OF TUMORS.

In my former experiments I made some observations on the influence of bacteria on the growth of tumors, and this series of transplantations has confirmed those former results It could be shown:

(*a*) That excised pieces of tumor so much infected with bacteria that later on ulceration of the growing tumor and coagulation of the cystic fluid took place, can lead to the formation of tumors.

(*b*) That if the infection reaches a certain point of severity no growth takes place, or that, in other cases, if the infection was not severe enough to prevent the growth in the beginning it will do so later on, if the cell degeneration in the center of the tumor had further advanced.

(*c*) From tumors which have ulcerated at one place frequently small well preserved nodules of sarcomatous tissue are separated and afterward are found lying free in the surrounding connective tissue.

(*d*) That pieces of tumor, after previous ulceration, may be able, by sloughing off of necrotic parts, to become covered by healthy tissue and that, in this way, sometimes multiple spheric nodules are formed, and that under these conditions these nodules frequently do not grow at all, or only grow slowly.

3.—ON THE INJECTION OF TUMOR JUICE.

By injection of the cystic fluid of tumors derived from the first cystic sarcoma, into the peritoneal cavity of

rats, I succeeded in four cases in producing the formation of sarcoma.

The second tumor, the sarcocarcinoma of the thyroid, contained none or only small cysts. In several cases, however, during this series of experiments, a small quantity of tumor fluid was injected without any result.

The third tumor again, contained like the first one, many large cysts and the transplanted pieces also developed cysts. In several cases a few cubic centimeters of the cystic fluid were injected into the peritoneal cavity of rats but without any success. In this connection another fact might be mentioned which may have some relation to this result: In the first series of transplantations, at the place where the tumors were introduced into the subcutaneous tissue, or into the peritoneal cavity of animals, isolated nodules of sarcoma frequently developed. The mere contact of the transplanted piece with the surface of the wound, was evidently sufficient to bring about this result.

In the second series of transplantation such a formation of sarcomatous nodules after transitory contact during the introduction of a piece was very rare indeed.

In the third series it almost never occurred, although these tumors, again like the tumors of the first series, were cystic.

This is an experimental proof of the fact that different tumors of similar structure may have a very different faculty to infect other tissues by mere contact.

At various periods cases have been reported in which surgeons believed that contact metastases during operation had taken place. It is, however, difficult in any other than an experimental way, by transplanting the same tumor in many animals, to compare the faculty of different tumors to infect.[1] It is impossible at present to indicate with certainty the causes of the different behavior of different tumors. One difference between the cystic sarcomas of the first and the third series, consisted in the fact that in the first series cells dividing mitotically were found inside of the cysts; in the third series such cells were not present in the cysts. This fact may also explain the different results obtained by injection of cystic fluid in these two series.

---

[1] By "infection" is not meant that a microorganism must be at the base of tumor formation.

4.—ON THE INJECTION OF FILTRATES OF TUMOR TISSUE PREVIOUSLY MINCED IN .7% SODIUM CHLORID SOLUTION.

An important question was if it would be possible to produce tumor formation after having excluded the injection of tumor cells by previous filtration. In one of my former papers I reported upon an experiment which consisted in the injection of minced tumor filtrate through a Berkefeld filter. The result was negative.[1] The new experiments had the same result as the former ones, no tumors developing after the injection of tumor juice previously filtered through a Berkefeld filter.

Equally unsuccessful was the injection of a tumor suspension filtered through ordinary filter paper. The injection of the supernatant fluid of tumor suspension proved, however, successful in several cases. It would seem that the implantation of small masses of tumor cells, or of an agency connected with these tumor cells, is necessary for the production of tumors.

After all these experiments, it can with great probability be excluded that some microorganism capable of existing outside of the cell, and so small that it can be filtered through a Berkefeld filter, is the cause of these sarcomas. Such microorganisms however are, as is well known, responsible for several other pathologic processes, and according to the just published experiments of Marx and Sticker, they are the cause of contagious epithelioma of the fowl, in which proliferation of epithelium is taking place.[2]

V.—ON THE INJECTION OF HYALINE BODIES TO BE FOUND IN PIECES OF TUMORS KEPT OUTSIDE OF THE BODY.

Pieces of sarcomas kept outside of the body for several weeks frequently show under the microscope many hyaline bodies of different sizes. These globules have been believed to be microorganisms causing tumor formation. I injected the fluid containing large numbers

---

[1] My first experiments on the injection of filtrates of tumor juice in animals of the same species were made in 1899 in cattle affected by carcinoma. Here also the results were not followed by the formation of tumors. Simultaneous with my second series of transplantations of tumors, M. Herzog carried on similar experiments on the injection of filtered juice, equally with negative results (Journal of Medical Research, Vol. III, 1902).

[2] Deutsche medicinische Wochenschrift, December, 1902.

of these bodies derived from the sarcoma of rats, into healthy rats without any subsequent growth of tumors. It seems much more probable that these hyaline bodies are disintegration products of cells or nuclei.

The experiments recorded here only represent the beginning of a line of experimentation which is capable of further extension. At the present stage of these investigations it is therefore not necessary to form conclusions of too definite a character. It may, however, be not without value to state in a preliminary way to what conclusions these experiments seem to lead.

(*a*) A microorganism living outside of tumor cells and passing through the pores of the Berkefeld filter is not, in all probability, the cause of the formation of sarcoma in rats.

(*b*) A microorganism living outside of tumor cells and resembling organisms like the tubercle bacillus, or belonging to the class of blastomycetes, is probably not the cause of the formation of sarcoma. Such a microorganism would probably not be made absolutely ineffective by heating to 45° C. for half an hour. A microscopic examination of tumors not secondarily infected does not show any bacteria or blastomycetes. No giant cells characteristic of granulomas caused by the tubercle bacillus or by the blastomycetes can be found in these sarcomas.

(*c*) The following possibilities remain: The formation of sarcoma is caused by an organism living outside the tumor cells, possessing however, a similar sensitiveness to heat as do the tumor cells themselves. Such organisms would have to be, as we may conclude from the foregoing experiments, larger than red blood-corpuscles as they seem to be unable to pass, at least under ordinary circumstances, through filter paper.

But since a careful microscopic examination of the tumors showed no structures other than tumor cells and their products of degeneration, and since also injection of the hyaline bodies referred to had negative results, the existence of such organisms would seem to be improbable. Still, further experiments ought to be made before these can with certainty be excluded.

The remaining possibilities are that a very small microorganism not capable of living outside of tumor cells is present; and lastly, that a microorganism is not the cause of tumor formation. It would therefore be impossible to produce tumors by the injection of micro-

organisms alone, if a microorganism incapable of living outside of tumor cells is present or if no microorganism is the cause of tumor formation.[1]

Further, we may conclude from these experiments that no organism sensitive to cold can be the cause of tumor formation.[2]

Without going into any details here I may add some further results of these experiments:

(*a*) After transplantation of these tumors there does not exist any appreciable period of latency in the growth of the peripheral transplanted tumor cells. They begin to multiply in a very short time, almost at once. This period of latency, however, seems to be different in different series of tumor transplantations; it was short in Hanau's and long in Moreau's experiments. It is of importance to investigate the cause of the different behavior of different tumors in this respect.

(*b*) Serial sections have shown that apparently separate tumor nodules of sarcoma may be connected by rows of cells, just as Petersen showed the connection of apparently separated carcinomatous cell nests.

(*c*) Tumor nodules smaller than a pea which never did expand actively when examined microscopically after two months, showed an active mitotic cell proliferation which is in all probability neutralized by the corresponding destruction of cells. In my former investigations I have shown experimentally that by cutting out a piece of such nodules an active growth may be produced.

(*d*) Sarcomatous cells are phagocytic and may, for instance, take up extravasated red blood-corpuscles. These phagocytic cells may have an appearance very similar to the phagocytes which I found in the cavity of the Graafian follicles in the first stage of atresia. The corresponding follicular cells therefore need not necessarily be leukocytes, but may, just as the sarcomatous phagocytes, be connective tissue cells.

(*e*) It is an important problem under what conditions transplantation of normal tissues succeeds; about the conditions under which transplantations of tumors are successful hardly any investigations exist. It is not improbable that transplantation of sarcoma will be shown to be more frequently successful than that of car-

[1] Several investigators claim to have produced tumors by the injection of microorganisms alone.

[2] Schüller maintains for microorganisms described by him an extreme sensitiveness to a temperature lower than that of the body.

cinoma, although transplantations of different varieties of carcinoma have been successful.

(*f*) The result of transplantation of tumor cells as compared with transplantation of ordinary tissues seems to be a conclusive demonstration of the fact that mere displacement of adult tissue, if it plays any role at all in the production of tumors (which however is not probable), is certainly quite an unessential factor.

(*g*) The fact that not infected, fresh pieces of sarcoma can be successfully transplanted into the majority of animals of the same species proves that a predisposition for the growth of tumor cells is not the main factor which prevents or favors the formation of metastases.

(*h*) Transplantation of tumor cells through so many generations would seem to prove that the life of ordinary tissue cells may, under conditions not realized in one organism, be able to live much longer than the individual to which they belong.

American Medicine

FOUNDED, OWNED AND CONTROLED BY THE MEDICAL PROFESSION OF AMERICA

GEORGE M. GOULD, Editor
G. C. C. HOWARD, Managing Editor
MARTIN B. TINKER, CHARLES S. DOLLEY } Assistant Editors

Subscription, $5 a year
Advertisements limited to 44 pages